Amendment

In the specification:

Please replace the first two paragraphs of the specification, below the heading "Cross-Reference to Related Applications," with the following paragraphs:

This application is a continuation of copending U.S. Serial No. 09/073,538, filed 6 May 1998, which is a continuation-in-part of copending U.S. Serial No. 08/846,247, filed 30 April 1997, which is a continuation-in-part of U.S. Serial No. 08/486,645, filed 7 June 1995, now U.S. Patent No. 5,712,146, which is continuation-in-part of U.S. Serial No. 08/238,811, filed 6 May 1994, now U.S. Patent No. 5,672,491, which is a continuation-in-part of U.S. Serial No. 08/164,301, filed 8 December 1993, now abandoned, which is a continuation-in-part of U.S. Serial No. 08/123,732, filed 20 September 1993, now abandoned. Priority is claimed under 35 USC §120. ~~Priority is also claimed under 35 USC §119(e) with respect to U.S. Serial No. 60/076,919, filed 5 March 1998, now lapsed.~~ The disclosures of these applications are incorporated herein by reference.

This application is also a continuation-in-part of copending U.S. Serial No. 09/311,756, filed 14 May 1999, which is a continuation of U.S. Serial No. 09/164,306, filed 1 October 1998, now abandoned, which claims benefit of priority to PCT Application No. PCT/US98/14911, filed 17 July 1998, which claims benefit of priority to U.S. Serial No. 08/896,323, filed 17 July 1997, now U.S. Patent No. 6,066,721, which is a continuation-in-part of U.S. Serial No. 08/675,817, filed 5 July 1996, now U.S. Patent No. 6,080,555, which claims benefit of priority under 35 USC 119(e) to U.S. Serial No. 60/003,338, filed 6 July 1995, now lapsed. The disclosures of these applications are incorporated herein by reference.

Please replace the paragraph beginning on page 1, line 30 with the following rewritten paragraph:

A number of lactones of keto acids have been synthesized using standard organic chemistry. These include a series of unsaturated ketolactones synthesized by Vedejes *et al.*, *J. Am Chem Soc* (1987) 109:5437-5446, shown as formulas 201, 202 and 203 in Figure 11 herein. Additional compounds of formulas 204 and 205, also shown in Figure 11 were synthesized as reported by Vedejes *et al. J Am Chem Soc* (1989) 111:8430-8438. In addition, compounds 206-208 (Figure 11) were synthesized by Borowitz, ~~(1975)~~; Mass spectra of 6-ketononanolides and related ketolactones, *J. Heterocyclic Chem.* 12(1):101-106; compound 209 has been synthesized by Ireland *et al.*, *J Org Chem* (1980) 45:1868-1880.

Please replace the paragraph beginning on page 2, line 28 with the following rewritten paragraph:

The PKS for erythromycin, used as an illustrative system is a modular PKS. Erythromycin was originally isolated from *S. erythraeus* (since reclassified as *Saccharopolyspora* ~~*erythrea*~~ *erythraea*) which was found in a soil sample from the Philippine archipelago. Cloning the genes was described by Donadio, S. *et al., Science* (1991) *252*:675. The particulars have been reviewed by Perun, T.J. in *Drug Action and Drug Resistance in Bacteria*, Vol. 1, S. Mitsuhashi (ed.) University Park Press, Baltimore, 1977. The antibiotic occurs in various glycosylated forms, designated A, B, ~~and~~ C, and D during various stages of fermentation. The entire erythromycin biosynthetic gene cluster from *S. erythraeus* has been mapped and sequenced by Donadio *et al.* in *Industrial Microorganisms: Basic and Applied Molecular Genetics* (1993) R.H. Baltz, G.D. Hegeman, and P.L. Skatrud (eds.) (*Amer Soc Microbiol*) and the entire PKS is an assembly of three such

multifunctional proteins usually designated DEBS-1, DEBS-2, and DEBS-3, encoded by three separate genes.

Please replace the paragraph beginning on page 3, line 9 with the following rewritten paragraph:

Expression of the genes encoding the PKS complex may not be sufficient to permit the production by the synthase enzymes of polyketides when the genes are transformed into host cells that do not have the required auxiliary phosphopantetheinyl transferase enzymes which posttranslationally modify the ACP domains of the PKS. Genes encoding some of these transferases are described in WO97/13845. In addition, enzymes that mediate glycosylation of the polyketides synthesized are described in WO97/23630. U.S. Serial No. 08/989,332 filed 11 December 1997, now U.S. Patent No. 6,033,883, describes the production of polyketides in hosts that normally do not produce them by supplying appropriate phosphopantetheinyl transferase expression systems. The contents of this application are incorporated herein by reference.

Please replace the paragraph beginning on page 3, line 20 with the following rewritten paragraph:

There have been attempts to alter the polyketide synthase pathway of modular PKS clusters. For example, European application 238,323 describes a process for enhancing production of polyketides by introducing a rate-limiting synthase gene and U.S. Patent No. 5,514,544 describes use of an activator protein for the synthase in order to enhance production. U.S. Patent Nos. 4,874,748 and 5,149,639 describe shuttle vectors that are useful in cloning modular PKS genes in general. Methods of introducing an altered gene into a microorganism chromosome are described in WO93/13663. Modification of the loading module for the DEBS-1 protein of the erythromycin-producing polyketide synthase to substitute the loading module for the avermectin-producing

polyketide synthase in order to vary the starter unit was described by Marsden, Andrew F.A. *et al. Science* (1998) 279:199-202 and Oliynyk, M. *et al. Chemistry and Biology* (1996) 3:833-839. WO 98/01571, published 15 January 1998, describes manipulation of the erythromycin PKS and ~~novel~~ polyketides resulting from such manipulation. In addition, WO ~~98/0156~~ 98/01546, also published 15 January 1998 describes a hybrid modular PKS gene for varying the nature of the starter and extender units to synthesize ~~novel~~ polyketides.

Please replace the paragraph beginning on page 4, line 4 with the following rewritten paragraph:

In addition, U.S. Patent Nos. 5,063,155 and 5,168,052 describe preparation of ~~novel~~ antibiotics using modular PKS systems. A number of modular PKS have been cloned. See, e.g., U.S. Patent No. 5,098,837, EP 791,655, EP 791,656 and U.S. Patent No. 5,252,474.

Please replace the paragraph beginning on page 5, line 29 with the following rewritten paragraph:

Figure 1A is a diagram of the erythromycin PKS complex from *S. erythraeus* showing the function of each multifunctional protein, and also shows the structure of ~~6dEB~~ 6-deoxyerythronolide B and of D-desosamine and L-cladinose.

Please replace the paragraph beginning on page 6, line 14 with the following rewritten paragraph:

Figures 8A and 8B shows antibiotics obtained from ~~the polyketides of~~ selected polyketides shown in Figures 6A-6F.

Please replace the paragraph beginning on page 6, line 15 with the following rewritten paragraph:

Figure 9 shows ~~the preparation of~~ a polyketide containing an unsaturated starter moiety and the corresponding antibiotic.

Please replace the paragraph beginning on page 6, line 24 with the following rewritten paragraph:

~~Modes of Carrying Out the Invention~~ Detailed Description of the Invention

Please replace the paragraph beginning on page 7, line 17 with the following rewritten paragraph:

The product in this case is 6dEB; the structure and numbering system for this molecule are shown in Figure 1A. Conversion to the antibiotics erythromycin A, B, C and D would require glycosylation generally by D-desosamine or L-mycarose, which ~~may ultimately be converted to cladinose at appropriate locations~~ is converted to cladinose in erythromycins A and B. Figure 1B diagrams the post-PKS biosynthesis of the erythromycins through addition of glycosyl groups.

Please replace the paragraph beginning on page 7, line 23 with the following rewritten paragraph:

As shown, 6dEB is converted by the gene *eryF* to erythronolide B which is, in turn, glycosylated by *eryB* to obtain 3-O-mycarosylerythronolide B which contains L-mycarose at ~~position 3~~ C-3. The enzyme *eryC* then converts this compound to erythromycin D by glycosylation with D-desosamine at ~~position 5~~ C-5. Erythromycin D, therefore, differs from 6dEB through glycosylation and by the addition of a hydroxyl group at ~~position 6~~ C-6. Erythromycin D can be converted to erythromycin B in a reaction catalyzed by *eryG* by methylating the L-mycarose residue at ~~position 3~~ C-3. Erythromycin D is converted to erythromycin C by the addition of a hydroxyl group at ~~position 12~~ C-12. Erythromycin A is obtained from erythromycin C by methylation of the mycarose residue catalyzed by *eryG*. The series of erythromycin antibiotics, then, differs by the level of hydroxylation of the polyketide framework and by the methylation status of the glycosyl residues.

Please replace the paragraph beginning on page 8, line 12 with the following rewritten paragraph:

The three DEBS-1, 2 and 3 proteins are encoded by the genetic segments ~~ery-AI, ery-AII and ery-AIII~~ *eryAI, eryAII and eryAIII*, respectively. These reading frames are located on the bacterial chromosome starting at about 10 kb distant from the erythromycin resistance gene ~~(ermE or eryR)~~ (*ermE* or *eryR*).

Please replace the paragraph beginning on page 9, line 5 with the following rewritten paragraph:

By a modular PKS "derived from" the erythromycin or other naturally occurring PKS is meant a modular polyketide synthase (or its corresponding encoding gene(s)) that retains the scaffolding of all of the utilized portion of the naturally occurring gene. (Not all modules need be included in the constructs.) On the constant scaffold, at least one enzymatic activity is mutated, deleted or replaced, so as to alter the activity. Alteration results when these activities are deleted or are replaced by a different version of the activity, or simply mutated in such a way that a polyketide other than the natural product results from these collective activities. This occurs because there has been a resulting alteration of the starter unit and/or extender unit, and/or stereochemistry, and/or chain length or cyclization and/or reductive or dehydration cycle outcome at a corresponding position in the product polyketide. Where a deleted activity is replaced, the origin of the replacement activity may come from a corresponding activity in a different naturally occurring polyketide synthase or from a different region of the same PKS. In the case of erythromycin, for example, any or all of the DEBS-1, DEBS-2 and DEBS-3 proteins may be included in the derivative or portions of any of these may be included; but the scaffolding of an erythromycin PKS protein is retained in whatever derivative is considered. Similar comments pertain to the corresponding ~~ery-AI, ery-AII and ery-AIII~~ *eryAI, eryAII and eryAIII* genes.

Please replace the paragraph beginning on page 10, line 5 with the following rewritten paragraph:

Thus, there are five degrees of freedom for constructing a polyketide synthase in terms of the polyketide that will be produced. First, the polyketide chain length will be determined by the

number of modules in the PKS. Second, the nature of the carbon skeleton of the PKS will be determined by the specificities of the acyl transferases which determine the nature of the extender units at each position -- e.g., malonyl, ~~methyl malonyl, or ethyl malonyl~~ methylmalonyl, or ethylmalonyl, *etc*. Third, the loading domain specificity will also have an effect on the resulting carbon skeleton of the polyketide. Thus, the loading domain may use a different starter unit, such as acetyl, propionyl, and the like. Fourth, the oxidation state at various positions of the polyketide will be determined by the dehydratase and reductase portions of the modules. This will determine the presence and location of ketone, alcohol, double bonds or single bonds in the polyketide. Finally, the stereochemistry of the resulting polyketide is a function of three aspects of the synthase. The first aspect is related to the AT/KS specificity associated with substituted malonyls as extender units, which affects stereochemistry only when the reductive cycle is missing or when it contains only a ketoreductase since the dehydratase would abolish chirality. Second, the specificity of the ketoreductase will determine the chirality of any β-OH. Finally, the enoyl reductase specificity for substituted malonyls as extender units will influence the result when there is a complete KR/DH/ER available.

Please replace the paragraph beginning on page 10, line 24 with the following rewritten paragraph:

In the working examples below, the foregoing variables for varying loading domain specificity which controls the starter unit, a useful approach is to modify the KS activity in module 1 which results in the ability to incorporate alternative starter units as well as module 1 extended units. This approach was illustrated in PCT application US/96/11317, published 23 Jan. 1997, as WO 97/02358, wherein the KS-I activity was inactivated through mutation. Polyketide synthesis is then initiated by feeding chemically synthesized analogs of module 1 diketide products. Working examples of this aspect are also presented hereinbelow.

Please replace the paragraph beginning on page 12, line 12 with the following rewritten paragraph:

The derivatives of ~~the~~ a naturally occurring PKS can be prepared by manipulation of the relevant genes. A large number of modular PKS gene clusters have been mapped and/or sequenced,

including erythromycin, soraphen A, rifamycin, and rapamycin, which have been completely mapped and sequenced, and FK506 and oleandomycin which have been partially sequenced, and candicidin, avermectin, and nemadectin which have been mapped and partially sequenced. Additional modular PKS gene clusters are expected to be available as time progresses. These genes can be manipulated using standard techniques to delete or inactivate activity encoding regions, insert regions of genes encoding corresponding activities ~~form~~ from the same or different PKS system, or otherwise mutated using standard procedures for obtaining genetic alterations. Of course, portions of, or all of, the desired derivative coding sequences can be synthesized using standard solid phase synthesis methods such as those described by ~~Jaye~~ Jay, E., *et al., J Biol Chem* (1984) 259:6331 and which are available commercially from, for example, Applied Biosystems, Inc.

Please replace the paragraph beginning on page 14, line 8 with the following rewritten paragraph:

If replacement of a particular target region in a host polyketide synthase is to be made, this replacement can be conducted *in vitro* using suitable restriction enzymes or can be effected *in vivo* using recombinant techniques involving homologous sequences framing the replacement gene in a donor plasmid and a receptor region in a recipient plasmid. Such systems, advantageously involving plasmids of differing temperature sensitivities are described, for example, in PCT ~~application~~ publication WO 96/40968.

Please replace the paragraph beginning on page 14, line 27 with the following rewritten paragraph:

Suitable control sequences include those which function in eucaryotic and procaryotic host cells. Preferred ~~host~~ hosts include fungal systems such as yeast and procaryotic hosts, but single cell cultures of, for example, mammalian cells could also be used. There is no particular advantage, however, in using such systems. Particularly preferred are yeast and procaryotic hosts which use control sequences compatible with *Streptomyces spp.* Suitable controls sequences for single cell cultures of various types of organisms are well known in the art. Control systems for expression in yeast, including controls which effect secretion are widely available ~~are~~ and routinely used. Control elements include promoters, optionally containing operator sequences, and other elements

depending on the nature of the host, such as ribosome binding sites. Particularly useful promoters for procaryotic hosts include those from PKS gene clusters which result in the production of polyketides as secondary metabolites, including those from aromatic (Type II) PKS gene clusters. Examples are *act* promoters, *tcm* promoters, spiramycin promoters, and the like. However, other bacterial promoters, such as those derived from genes that encode sugar metabolizing enzymes, such as galactose, lactose (*lac*) and maltose, are also useful. Additional examples include promoters derived from genes that encode biosynthetic enzymes for compounds such as tryptophan (*trp*), and the β-lactamase (*bla*), bacteriophage lambda PL, and T5 promoters. In addition, synthetic promoters, such as the *tac* promoter (U.S. Patent No. 4,551,433), can be used.

Please replace the paragraph beginning on page 16, line 5 with the following rewritten paragraph:

As described above, particularly useful control sequences are those which themselves, or using suitable regulatory systems, activate expression during transition from growth to stationary phase in the vegetative mycelium. The system contained in the illustrated plasmid pCK7, i.e., the *act*I/*act*III promoter pair and the *act*II-ORF4, an activator gene, is particularly preferred. Particularly preferred hosts are those which lack their own means for producing polyketides so that a cleaner result is obtained. Illustrative host cells of this type include the modified *S. coelicolor* CH999 culture described in PCT ~~application~~ publication WO 96/40968 and similar strains of *S. lividans*.

Please replace the paragraph beginning on page 16, line 13 with the following rewritten paragraph:

The expression vectors containing nucleotide sequences encoding a variety of PKS systems for the production of different polyketides are then transformed into the appropriate host cells to construct the library. In one straightforward approach, a mixture of such vectors is transformed into the selected host cells and the resulting cells plated into individual colonies and selected for successful transformants. Each individual colony will then represent a colony with the ability to produce a particular PKS synthase and ultimately a particular polyketide. Typically, there will be duplications in some of the colonies; the subset of the transformed colonies that contains a different

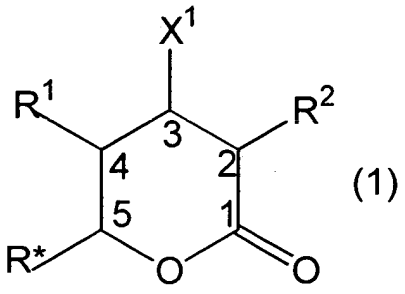
PKS in each member colony can be considered the library. Alternatively, the expression vectors can be used individually to transform hosts, which transformed hosts are then assembled into a library. A variety of strategies might be devised to obtain a multiplicity of colonies each containing a PKS gene cluster derived from the naturally occurring host gene cluster so that each colony in the library produces a different PKS and ultimately a different polyketide. The number of different polyketides that are produced by the library is typically at least four, more typically at least ten, and preferably at least 20, more preferably at least 50, reflecting similar numbers of different altered PKS gene clusters and PKS gene products. The number of members in the library is arbitrarily chosen; however, the degrees of freedom outlined above with respect to the variation of starter, extender units, stereochemistry, oxidation state, and chain length ~~is quite large~~ allow quite large libraries.

Please replace the paragraph beginning on page 18, line 4 with the following rewritten paragraph:

Indeed, a large number of novel polyketides have been prepared according to the method of the invention as illustrated in the examples below. These novel polyketides are useful intermediates in formation of compounds with antibiotic activity through glycosylation reactions as described above. As indicated above, the individual polyketides are reacted with suitable sugar derivatives to obtain compounds ~~of~~ with antibiotic activity. Antibiotic activity can be verified using typical screening assays such as those set forth in Lehrer, R. *et al. J Immunol Meth* (1991) 137:167-173.

Please replace the paragraph beginning on page 18, line 14 with the following rewritten paragraph:

In one embodiment, the polyketides of the invention include the compounds of structure (1) and the glycosylated forms thereof. ~~Thecompounds~~ The compounds include the polyketide structure:



(1)

including the isolated stereoisomeric forms thereof;

wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-15C;

each of R^1 and R^2 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

X^1 is H_2, HOH or =O;

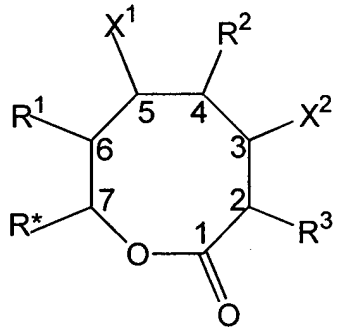
with the provisos that:

at least one of R^1 and R^2 must be alkyl (1-4C); and

the compound is other than compounds 1, 2, 3, 5 and 6 of Figure 6A.

Please replace the paragraph beginning on page 19, line 1 with the following rewritten paragraph:

In another embodiment, the polyketides of the invention include the compounds of formula (2) and the glycosylated forms thereof. These compounds include the polyketide structure:



(2)

including the isolated stereoisomeric forms thereof;

wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-15C;

each of R^1, R^2 and R^3 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

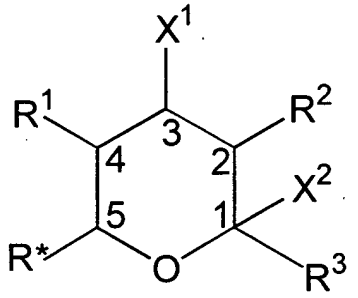
each of X^1 and X^2 is independently H_2, HOH or =O;

with the provisos that:

at least two of R^1, R^2 and R^3 are alkyl (1-4C).

Please replace the paragraph beginning on page 19, line 13 with the following rewritten paragraph:

In another embodiment, the polyketides of the invention include the compounds of structure (3) and the glycosylated forms thereof. ~~Thecompounds~~ The compounds include the polyketide structure:



(3)

including the isolated stereoisomeric forms thereof;

wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-15C;

each of R^1, R^2 and R^3 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

each of X^1 and X^2 is independently H_2, HOH or =O;

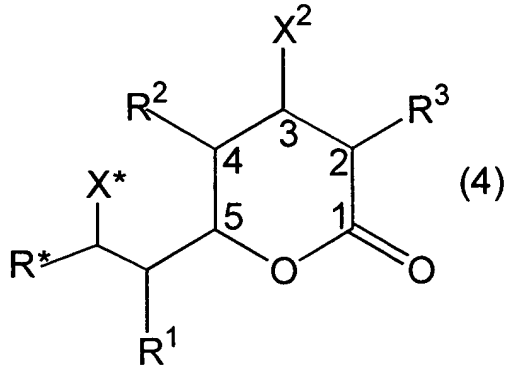
with the provisos that:

at least one of R^1 and R^2 must be alkyl (1-4C); and

the compound is other than compound 8 of Figure 6A.

Please replace the paragraph beginning on page 20, line 6 with the following rewritten paragraph:

Still other embodiments are those of the following formula, including the glycosylated forms thereof. These are derived from the compound of formula (4) which has the structure:



including the isolated stereoisomeric forms thereof;

wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-15C;

each of R^1, R^2 and R^3 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

each of X* and X^2 is independently H_2, HOH or =O;

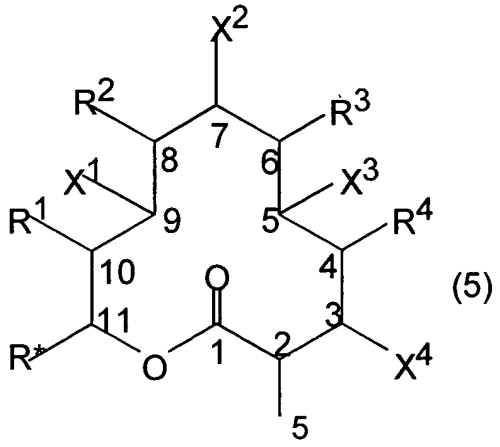
with the provisos that:

at least one of R^2 and R^3 is alkyl (1-4C); and

the compound is other than compound 9 of Figure 6A.

Please replace the paragraph beginning on page 20, line 19 with the following rewritten paragraph:

Still other embodiments are the result of the condensation of five modules of the polyketide synthase system. The polyketide forms of these compounds are of the formula:



including the glycosylated and isolated stereoisomeric forms thereof;

wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-15C;

each of R^1, R^2, R^3, R^4 and R^5 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

each of X^1, X^2, X^3 and X^4 is independently H_2, HOH or =O; or

X^1 or X^2 or X^3 or X^4 is H and the compound of formula (5) contains a π-bond at positions 8-9 or 6-7 or 4-5 or 2-3;

with the provisos that:

at least two of R^1-R^5 are alkyl (1-4C); and

the compound is other than compound 13 or 14 of Figure 6A or compound 205, 210-213 of ~~figure~~ Figure 11.

Please replace the paragraph beginning on page 21, line 14 with the following rewritten paragraph:

Preferred forms of compounds ~~that include formula (5) as~~ of formula (5) are those wherein at least three, more preferably at least four, of R^1-R^5 are alkyl (1-4C), preferably methyl or ethyl.

Please replace the paragraph beginning on page 21, line 17 with the following rewritten paragraph:

Also preferred are compounds wherein X^1 is -OH and/or ~~X^2 =O~~ X^2 is =O, and/or X^3 is H.

Please replace the paragraph beginning on page 22, line 17 with the following rewritten paragraph:

Also preferred are those wherein X^2 is H_2, =O or H▶···OH, ~~and/or X^3~~ and/or X^3 is H, and/or X^1 is OH and/or X^4 is OH and/or X^5 is OH.

Please replace the paragraph beginning on page 26, line 27 with the following rewritten paragraph:

In more detail, pCK7 ~~(Figure 4)~~ Figure 3, a shuttle plasmid containing the complete *eryA* genes, which were originally cloned from pS1 (Tuan *et al. Gene* (1990) 90:21), was constructed as

follows. The modular DEBS PKS genes were transferred incrementally from a temperature-sensitive "donor" plasmid, i.e., a plasmid capable of replication at a first, permissive temperature and incapable of replication at a second, non-permissive temperature, to á "recipient" shuttle vector via a double recombination event, as depicted in ~~Figure 5~~ Figure 4. A 25.6 kb *Sph*I fragment from pS1 was inserted into the *Sph*I site of pMAK705 (Hamilton *et al. J Bacteriol* (1989) 171:4617) to give pCK6 (Cm^R), a donor plasmid containing *eryAII, eryAIII,* and the 3' end of *eryAI.* Replication of this temperature-sensitive pSC101 derivative occurs at 30°C but is arrested at 44°C. The recipient plasmid, pCK5 (Ap^R, Tc^R), includes a 12.2 kb *eryA* fragment from the *eryAI* start codon (Caffrey *et al. FEBS Lett* (1992) 304:225) to the *Xcm*I site near the beginning of *eryAII*, a 1.4 kb *EcoR*I-*Bsm*I pBR322 fragment encoding the tetracycline resistance gene (*Tc*), and a 4.0 kb *Not*I-*EcoR*I fragment from the end of *eryAIII. Pac*I, *Nde*I, and ribosome binding sites were engineered at the *eryAI* start codon in pCK5. pCK5 is a derivative of pRM5 (described above). The 5' and 3' regions of homology are 4.1 kb and 4.0 kb, respectively. MC1061 *E. coli* was transformed with pCK5 and pCK6 and subjected to carbenicillin and chloramphenicol selection at 30°C. Colonies harboring both plasmids (Ap^R, Cm^R) were then restreaked at 44°C on carbenicillin and chloramphenicol plates. Only cointegrates formed by a single recombination event between the two plasmids were viable. Surviving colonies were propagated at 30°C under carbenicillin selection, forcing the resolution of the cointegrates via a second recombination event. To enrich for pCK7 recombinants, colonies were restreaked again on carbenicillin plates at 44°C. Approximately 20% of the resulting colonies displayed the desired phenotype (Ap^R, Tc^S, Cm^S). The final pCK7 candidates were thoroughly checked via restriction mapping. A control plasmid, pCK7f, which contains a frameshift error in *eryAI*, was constructed in a similar manner. pCK7 and pCK7f were transformed into *E. coli* ET12567 (MacNeil *J Bacteriol* (1988) 170:5607) to generate unmethylated plasmid DNA and subsequently moved into *Streptomyces coelicolor* CH999.

Please replace the paragraph beginning on page 28, line 10 with the following rewritten paragraph:

For each of the six modules of DEBS, a subclone was made containing endonuclease restriction sites engineered at selected boundaries of the acyltransferase (AT) and reduction (KR or DH/ER/KR) domains. The restriction sites were introduced into the subclones by PCR mutagenesis.

A *Bam*HI site was used for the 5' boundary of AT domains, a *Pst*I site was introduced between the AT and reductive domains, and *Xba*I was used at the 3' end of the reductive domain (see Figure 5). This resulted in the following engineered sequences (~~lowercase~~ lower case indicates engineered restriction site) (SEQ ID NOS:1-18, in order of appearance):

Module 1 (pKOS011-16)

5' AT boundary	GCGCAGCAG*ggatcc*GTCTTCGTC
AT/KR boundary	CGCGTCTGG*ctgcag*CCGAAGCCG
3' KR boundary	CCGGCCGAA*tctaga*GTGGGCGCG

Module 2 (pKOS001-11)

5' AT boundary	TCCGACGGT*ggatcc*GTGTTCGTC
AT/KR boundary	CGGTTCTGG*ctgcag*CCGGACCGC
3' KR boundary	ACGGAGAGC*tctaga*GACCGGCTG

Module 3 (pKOS024-2)

5' AT boundary	GACGGGCGC*ggatcc*GTCTTCCTG
AT/KR boundary	CGCTACTGG*ctgcag*CCCGCCGCA
3' KR boundary	ACCGGCGAG*tctaga*CAACGGCTC

Module 4 (pKOS024-3)

5' AT boundary	GCGCCGCGC*ggatcc*GTCCTGGTC
AT(DH/ER/KR) boundary	CGCTTCTGG*ctgcag*CCGCACCGG
3' DH/ER/KR boundary	GGGCCGAAC*tctaga*GACCGGCTC

Module 5 (pKOS006-182)

5' AT boundary	ACTCGCCGC*ggatcc*GCGATGGTG
AT/KR boundary	CGGTACTGG*ctgcag*ATCCCCACC
3' KR boundary	GAGGAGGGC*tctaga*CTCGCCCAG

Module 6 (pKOS015-52)

5' AT boundary	TCCGCCGGC*ggatcc*GTTTTCGTC
AT/KR boundary	CGGTACTGG*ctgcag*CCGGAGGTG
3' KR boundary	GTGGGGGCC*tctaga*GCGGTGCAG

Please replace the paragraph beginning on page 30, line 4 with the following rewritten paragraph:

The following are typical procedures. The products are indicated by their numbers in Figure 6, as well as listed in Table 2, below, where "a" represents the embodiment where R is methyl; "b" represents the embodiment where R is hydrogen.

Please replace the paragraph beginning on page 30, line 7 with the following rewritten paragraph:

a) Replacement of DEBS DH/ER/KR4. A portion of the erythromycin gene of module 4 (eryDH/ER/KR4) was replaced either with the corresponding rapamycin activities of the first rapamycin module (rapDH/ER/KR1) or of module 4 of rapamycin (rapDH/KR4). The replacement utilized the technique of Kao *et al. Science* (1994) 265:509-512. A donor plasmid was prepared by first amplifying 1 kbp regions flanking the DH/ER/KR4 of DEBS to contain a *Pst*I site at the 3' end of the left flank and an *Xba*I site at the 5' end of the right flank. The fragments were ligated into a temperature-sensitive donor plasmid, in a manner analogous to that set forth for KR6 in paragraph b) of this example, and the rapamycin cassettes prepared as described in Example 2 were inserted into the *Pst*I/*Xba*I sites. The recipient plasmid was pCK7 described in Preparation A. The *in vivo* recombination technique resulted in the expression plasmid pKOS011-19 (eryDH/ER/KR4 → rapDH/ER/KR1) and pKOS011-21 (eryDH/ER/KR4 → rapDH/KR4). The junctions at which the *Pst*I and *Xba*I sites were introduced into DEBS in both vectors are as follows:

GAGCCCCAGCGGTACTGGCTGCAG rap cassette
TCTAGAGCGGTGCAGGCGGCCCCG (SEQ ID NOS:32-33)

Please replace the paragraph beginning on page 31, line 2 with the following rewritten paragraph:

The resulting expression vectors were transformed into *S. coelicolor* CH999 and successful transformants grown as described above. The transformant containing the rapDH/ER/KR1 cassette produced the polyketide shown in ~~Figure 6 as 23a,b~~ Figure 6 as compound 23 and listed in Table 2 as 23a,b; the transformant containing the plasmid with rapDH/KR4 cassette produced the polyketide shown in ~~Figure 6 as 24a,b~~ Figure 6 as compound 24 and listed in Table 2 as 24a,b . As shown,

these polyketides differ from 6-deoxyerythronolide B by virtue of a 6,7 alkene in the case of 24a and by the C6-methyl stereochemistry in the case of 23a.

Please replace the paragraph beginning on page 31, line 28 with the following rewritten paragraph:

Transformants of *S. coelicolor* CH999 resulted in the production of the polyketide shown in ~~Figure 6 as 74a,b~~ Figure 6 as compound 74 and listed in Table 2 as 74a,b.

Please replace the paragraph beginning on page 31, line 30 with the following rewritten paragraph:

c) Replacement of DEBS KR2. The eryKR2 enzymatic activity was replaced in a series of vectors using *in vitro* insertion into the *Pst*I/*Xba*I sites of pKAO263. pKAO263 is a derivative of pCK13 described in Kao, C.M. *J Am Chem Soc* (1996) 118:9184-9185. It was prepared by introducing the *Pst*I and *Xba*I restriction sites positioned identically to those in the analogous 2-module DEBS system described by Bedford, D. *et al. Chem an Biol* (1996) 3:827-831. Three expression plasmids were prepared: pKOS009-7 (eryKR2 → rapDH/KR4); pKAO392 (eryKR2 → rapKR2); and pKAO410 (eryKR2 → rapDH/ER/KR1). ~~these~~ These plasmids, when transformed into *S. coelicolor* CH999, resulted in the production of polyketides with the structures 12a,b; 3a,b; and 10a, 11a,b ~~in Figure~~ listed in Table 2 and shown in Figure 6, respectively. An additional vector, pKAO400 (eryKR2 → rapKR4) produced the same results as pKAO392.

Please replace the paragraph beginning on page 32, line 20 with the following rewritten paragraph:

S. coelicolor CH999 transformed with the resulting plasmid, pKOS008-51, produced the polyketides 6a,b shown in Figure 6 as structure 6.

Please replace the paragraph beginning on page 32, line 25 with the following rewritten paragraph:

The following is a typical procedure. The products are indicated by their numbers in Figure 6, and listed in Table 2, where "a" represents the embodiment where R is methyl; "b" represents the embodiment where R is hydrogen.

Please replace the paragraph beginning on page 32, line 28 with the following rewritten paragraph:

A duplex oligonucleotide linker (ΔRdx) was designed to allow complete excision of reductive cycle domains. Two synthetic oligonucleotides (SEQ ID NOS:42-43):

5'-GCCGGACCGCACCACCCCTCGTGACGGAGAACCGGAGACGGAGAGCT-3'
3'-ACGTCGGCCTGGCGTGGTGGGGAGCACTGCCTCTTGGCCTCTGCCTCTCGAGATC-5'
*Pst*I *Xba*I

were designed to generate *Pst*I- and *Xba*I-compatible ends upon hybridization. This duplex linker was ligated into the *Pst*I- and *Xba*I-sites of the recombination donor plasmid containing the appropriate left- and right-flanking regions of the reductive domain to be excised. The *in vivo* recombination technique of Example 3, paragraph a) was then used. The donor plasmid contained the duplex linker ΔRdx having a *Pst*I and *Xba*I compatible end ligated into the *Pst*I and *Xba*I sites of the plasmid modified to contain the left and right flanking regions of the reductive domain to be excised. The donor plasmids were recombined with recipient plasmid pCK7 to generate pKOS011-13 (eryKR6 → ΔRdx) and with recipient plasmid pCK13 to obtain pKOS005-4 (eryKR2 → ΔRdx). When transformed into *S. coelicolor* CH999, plasmid pKOS011-13 produced the polyketides ~~30a,b, 31a,b, 77a,b and 78a,b;~~ listed in Table 2 as 30a,b, 31a,b, 77a,b and 78a,b and shown in Figure 6 as structures 30, 31, 77, and 78; ~~in Figure 6 plasmid pKOS005-4 produced the polyketide 2a,b~~ and plasmid pKOS005-4 produced the polyketide listed in Table 2 as 2a,b, and shown in Figure 6 as structure 2.

Please replace the paragraph beginning on page 33, line 16 with the following rewritten paragraph:

The following are typical procedures. The products are indicated by their numbers in Figure 6 and listed in Table 2, where "a" represents the embodiment where R is methyl; "b" represents the embodiment where R is hydrogen.

Please replace the paragraph beginning on page 33, line 19 with the following rewritten paragraph:

Using the expression system of Kao, C. M. *et al. Science* (1994) 265:509-512, the expression of DEBS1 alone (1 + 2), in the absence of DEBS2 and DEBS3 (in plasmid pCK9), resulted in the production of (2*R*,3*S*,4*S*,5*R*)-2,4-dimethyl-3,5-dihydroxy-*n*-heptanoic acid L-lactone ("the heptanoic acid L-lactone" ~~(1a)~~ (see ~~Figures 6 and 7~~ Figure 6, compound 1, and Figure 7A)) (1-3 mg/L), the expected triketide product of the first two modules (Kao, C. M. *et al. J Am Chem Soc* (1994) 116:11612-11613). Thus, a thioesterase is not essential for release of a triketide from the enzyme complex.

Please replace the paragraph beginning on page 34, line 27 with the following rewritten paragraph:

CH999/pCK12 produced the heptanoic acid L-lactone (1a) (20 mg/L) as determined by ^{1}H and ^{13}C NMR spectroscopy. This triketide product is identical to that produced by CH999/pCK9, which expresses the unmodified DEBS1 protein alone described above. However, CH999/pCK12 produced ~~6Ba~~ compound 1a (compound 1, where R is methyl, in Figure 6) in significantly greater quantities than did CH999/pCK9 (>10 mg/L vs. ~1 mg/L), indicating the ability of the TE to catalyze thiolysis of a triketide chain attached to the ACP domain of module 2. CH999/pCK12 also produced significant quantities of ~~1b~~ compound 1b, a novel analog of ~~1a~~ compound 1a, (10 mg/L), that resulted from the incorporation of an acetate start unit instead of propionate. This is reminiscent of the ability of CH999/pCK7, which expresses the intact PKS, to produce 8,8a-deoxyoleandolide (see Figure 1A) in addition to 6dEB described above.

Please replace the paragraph beginning on page 35, line 6 with the following rewritten paragraph:

Since ~~1b~~ compound 1b was not detected in CH999/pCK9, its facile isolation from CH999/pCK12 provides additional evidence for the increased turnover rate of DEBS1 due to the presence of the TE. In other words, the TE can effectively recognize an intermediate bound to a

"foreign" module that is four acyl units shorter than its natural substrate, 6dEB. However, since the triketide products can probably cyclize spontaneously into ~~1a and 1b~~ compounds 1a and 1b under typical fermentation conditions (pH 7), it is not possible to discriminate between a biosynthetic model involving enzyme-catalyzed lactonization and one involving enzyme-catalyzed hydrolysis followed by spontaneous lactonization. Thus, the ability of the 1+2+TE PKS to recognize the C-5 hydroxyl of a triketide as an incoming nucleophile is unclear.

Please replace the paragraph beginning on page 35, line 16 with the following rewritten paragraph:

CH999/pCK15, produced abundant quantities of (8*R*,9*S*)-8,9-dihydro-8-methyl-9-hydroxy-10-deoxymethonolide (compound 13 in Figure 6) (10 mg/L), demonstrating that the pentamodular PKS is active. Compound 13 was characterized using ^{1}H and ^{13}C NMR spectroscopy of natural abundance and ^{13}C-enriched material, homonuclear correlation spectroscopy (COSY), heteronuclear correlation spectroscopy (HETCOR), mass spectrometry, and molecular modeling. Compound 13 is an analog of 10-deoxymethonolide (compound 14, Lambalot, R.H. *et al. J Antibiotics* (1992) 45:1981-1982), the aglycone of the macrolide antibiotic methymycin. The production of ~~13~~ compound 13 by a pentamodular enzyme demonstrates that active site domains in modules 5 and 6 in DEBS can be joined without loss of activity. Thus, it appears that individual modules as well as active sites are independent entities which do not depend on association with neighboring modules to be functional. The 12-membered lactone ring, formed by esterification of the terminal carboxyl with the C-11 hydroxyl of the hexaketide product, indicated the ability of the 1+2+3+4+5+TE PKS, and possibly the TE itself, to catalyze lactonization of a polyketide chain one acyl unit shorter than the natural product of DEBS, 6dEB. Indeed, the formation of ~~the 13~~ compound 13 may mimic the biosynthesis of the closely related 12-membered hexaketide macrolide, methymycin, which frequently occurs with the homologous 14-membered heptaketide macrolides, picromycin and/or narbomycin (Cane, D. E. *et al. J Am Chem Soc* (1993) 115:522-566). The erythromycin PKS scaffold can thus be used to generate a wide range of macrolactones with shorter as well as longer chain lengths.

Please replace the paragraph beginning on page 36, line 26 with the following rewritten paragraph:

The XAD-16 resin was collected by vacuum filtration, washed with water, then extracted twice with 10 mL portions of ethanol. The extracts were combined and evaporated to a slurry, which was extracted with ethyl acetate. The ethyl acetate was washed once with sat. $NaHCO_3$ and evaporated to yield the crude product. Samples were dissolved in ethanol and analyzed by LC/MS. The HPLC used a 4.6 x 150 mm C18 reversed-phase column with a gradient from 80:19:1 $H_2O/CH_3CN/CH_3CO_2H$ to 99:1 CH_3CN/CH_3CO_2H. Mass spectra were recorded using a Perkin-Elmer/Sciex API100LC spectrometer fitted with an APCI ion source. Each genetic construct typically resulted in formation of products in pairs, indicated in ~~the Figure 6 and in~~ Table 2 by the letters "a" (R = CH3) and "b" (R = H), arising from priming of the PKS by and propionyl-CoA and acetyl-CoA, respectively.

Please replace the paragraph beginning on page 37, line 7 with the following rewritten paragraph:

Using the foregoing techniques, the DEBS constructs shown in Table 2 were prepared. The products obtained when the constructs were transformed into *S. coelicolor* CH999 are indicated by their numbers in Figure 6 and in Table 2, where "a" represents the embodiment where R is methyl; "b" represents the embodiment where R is hydrogen. Some of the expression vectors were prepared by *in vitro* ligation; multiple domain substitutions were created by subsequent *in vitro* ligations into the singly-substituted expression plasmids. Others were obtained by *in vivo* recombination.

Please replace Table 2 beginning on page 37 with the following rewritten Table 2:

Table 2

Plasmid	Modules	Domain Substitution	Products (see Figure 6)
***In Vitro* Ligation**			
KOS011-28	2	eryAT1 → rapAT2	4-nor-TKL (5a,b)
KOS008-51	2	eryAT2 → rapAT2	2-nor-TKL (6a,b)
KOS014-62	2	eryKR2 → rapDH/ER/KR1	3-deoxy-TKL (4a,b)
KAO410	3	eryKR2 →	KAO410 (10a,b)

Table 2			
Plasmid	Modules	Domain Substitution	Products (see Figure 6)
		rapDH/ER/KR1	3-deoxy-hemiketal (11a,b)
KAO392	3	eryKR2 → rapKR2	3-epi-TKL (3a,b)
KOS009-7	3	eryKR2 → rapDH/KR4	KOS009-7 (12a,b)
KOS015-30	6	eryAT3 → rapAT2	8-nor-6dEB (18a,b)
KOS016-47	6	eryAT5 → rapAT2	4-nor-6dEB (19a,b)
KOS026-18b	6	eryKR5 → rapDH/ER/KR1	5-deoxy-6dEB (26a,b)
KOS016-32	6	eryKR5 → rapDH/KR4	4,5-~~dehydro~~ anhydro-6dEB (27a,b)
KOS016-28	6	eryKR5 → ΔRdx	5-oxo-6dEB (28a,b)
KOS015-63	6	eryAT6 → rapAT2	2-nor-6dEB (20a,b)
KOS015-83	6	eryAT2 → rapAT2 + eryKR2 → rapDH/KR4	10-nor-10,11-~~dehydro~~ anhydro-6dEB (32a,b)
KOS015-84	6	eryAT2 → rapAT2 + eryKR2 → rapDH/ER/KR1	10-nor-11-deoxy-6dEB (33a,b)
KOS016-100	6	eryAT5 → rapAT2 + eryKR5 → Δrdx	4-nor-5-oxo-6dEB (38a,b)
KOS015-106	6	eryAT6 → rapAT2 + eryKR6 → rapKR2	2-nor-3-epi-6dEB (42a,b)
KOS015-109	6	eryAT6 → rapAT2 + eryKR6 → Δrdx	2-nor-3-oxo-6dEB (31a,b)
KOS011-90	6	eryAT2 → rapAT2 + eryKR5 → rapDH/KR4	4,5-~~dehydro~~ anhydro-10-nor-6dEB (34a,b)
KOS011-84	6	eryAT2 → rapAT2 + eryKR5 → Δrdx	5-oxo-10-nor-6dEB (35a,b)
KOS011-82	6	eryKR2 → rapDH/KR4 + eryAT5 → rapAT2	4-nor-10,11-~~dehydro~~ anhydro-6dEB (39a,b)
KOS011-85	6	eryKR2 → rapDH/KR4 + eryKR5 → Δrdx	5-oxo-10,11-~~dehydro~~ anhydro-6dEB (57a,b)
KOS011-87	6	eryKR2 → rapDH/KR4 + eryAT5 → rapAT2 + eryKR5 → Δrdx	4-nor-5-oxo-10,11-~~dehydro~~ anhydro- 6dEB (65a,b)
KOS011-83	6	eryKR2 →	4-nor-11-deoxy-6dEB (40a,b)

Table 2			
Plasmid	Modules	Domain Substitution	Products (see Figure 6)
		rapDH/ER/KR1 + eryAT5 → rapAT2	
KOS011-91	6	eryKR2 → rapDH/ER/KR1 + eryKR5 → rapDH/KR4	4,5-~~dehydro~~ anhydro-11-deoxy-6dEB (55a,b)
KOS011-86	6	eryKR2 → rapDH/ER/KR1 + eryKR5 → Δrdx	5-oxo-11-deoxy-6dEB (56a,b)
KOS011-88	6	eryKR2 → rapDH/ER/KR1 + eryAT5 → rapAT2 eryKR5 → Δrdx	4-nor-5-oxo-11-deoxy-6dEB (69a,b)
KOS015-40	6	eryAT2 → rapAT2 + eryKR6 → rapDH/KR4	2,3-~~dehydro~~ anhydro-10-nor-6dEB (76a,b)
KOS015-41	6	eryAT2 → rapAT2 + eryKR6 → Δrdx	3-oxo-10-nor-6dEB (36a,b) 10-nor-spiroketal (79a,b)
KOS015-44	6	eryKR2 → rapDH/ER/KR1 + eryAT6 → rapAT2	2-nor-11-deoxy-6dEB (45a,b)
KOS015-45	6	eryKR2 → rapDH/ER/KR1 + eryKR6 → RapDH/KR4	2,3-~~dehydro~~ anhydro-11-deoxy-6dEB (75a,b)
KOS015-46	6	eryKR2 → rapDH/ER/KR1 + eryKR6 → Δrdx	3-oxo-11-deoxy-6dEB (53a,b)
KOS015-42	6	eryKR2 → rapDH/KR4 + eryAT6 → rapAT2	2-nor-10,11-~~dehydro~~ anhydro-6dEB (46a,b)
KOS015-43	6	eryKR2 → rapDH/KR4 + eryKR6 → Δrdx	3-oxo-10,11-~~dehydro~~ anhydro-6dEB (54a,b)
KOS015-88	6	eryKR2 → rapDH/KR4 + eryKR6 → rapKR2	3-epi-10,11-~~dehydro~~ anhydro-6dEB (48a,b)
KOS015-89	6	eryKR2 → rapDH/ER/KR1 + eryKR6 → rapKR2	3-epi-11-deoxy-6dEB (49a,b)
KOS015-87	6	eryAT2 → rapAT2 + eryKR6 → rapKR2	3-oxo-10-nor-6dEB (36a,b)

Table 2			
Plasmid	Modules	Domain Substitution	Products (see Figure 6)
KOS015-117	6	eryAT2 → rapAT14 + eryAT6 → rapAT2	2,10-bisnor-6dEB (37a,b)
KOS015-120	6	eryAT2 → rapAT14 + eryAT6 → rapAT2 + eryKR6 → Δrdx	2,10-bisnor-3-oxo-6dEB (58a,b) 2,10-bisnor-spiroketal (80a,b)
KOS015-121	6	eryKR2 → rapDH/KR4 + eryAT6 → rapAT2 + eryKR6 → rapKR2	2-nor-3-epi-10,11-~~dehydro~~ anhydro-6dEB (62a,b)
KOS015-122	6	eryKR2 → rapDH/KR4 + eryAT6 → rapAT2 + eryKR6 → Δrdx	2-nor-3-oxo-10,11-~~dehydro~~ anhydro-6dEB (63a,b)
KOS015-123	6	eryKR2 → rapDH/ER/KR1 + eryAT6 → rapAT2 + eryKR6 → rapKR2	2-nor-3-epi-11-deoxy-6dEB (66a,b)
KOS015-125	6	eryKR2 → rapDH/ER/KR1 + eryAT6 → rapAT2 + eryKR6 → Δrdx	2-nor-3-oxo-11-deoxy-6dEB (67a,b)
KOS015-127	6	eryAT2 → rapAT2 + eryKR2 → rapDH/KR4 + eryKR6 → rapKR2	3-epi-10-nor-10,11-~~dehydro~~ anhydro-6dEB (64a,b)
KOS015-150	6	eryAT2 → rapAT2 + eryKR2 → rapDH/KR4 + eryAT6 → rapAT2	2,10-bisnor-10,11-~~dehydro~~ anhydro-6dEB (59a,b)
KOS015-158	6	eryAT2 → rapAT2 + eryKR2 → rapDH/ER/KR1 + eryKR6 → Δrdx	3-oxo-10-nor-11-deoxy-6dEB (68a,b)
KOS015-159	6	eryAT2 → rapAT2 + eryKR2 → rapDH/ER/KR1 + eryAT6 → rapAT2	2,10-bisnor-11-deoxy-6dEB (60a,b)
KOS016-133K	6	eryKR5 → rapDH/KR4 +	3-oxo-4,5-~~dehydro~~ anhydro-6dEB (51a,b)

Table 2			
Plasmid	Modules	Domain Substitution	Products (see Figure 6)
		eryKR6 → ΔrdX	3,5-dioxo-6dEB (52a,b)
KOS016-150B	6	eryKR5 → ΔrdX + eryKR6 → rapKR4	3-epi-5-oxo-6dEB (50a,b)
KOS016-183F	6	eryAT5 → rapAT2 + eryAT6 → rapAT2	2,4-bisnor-6dEB (41a,b)
KOS016-183G	6	eryAT5 → rapAT2 + eryAT6 → rapAT2 + eryKR6 → rapKR2	2,4-bisnor-3-epi-6dEB (61a,b)
KOS016-152E	6	eryKR5 → rapDH/KR4 + eryAT6 → rapAT2	2-nor-4,5-~~dehydro~~ anhydro-6dEB (43a,b)
KOS016-152F	6	eryKR5 → rapDH/KR4 + eryAT6 → rapAT2 + eryKR6 → rapKR2	2-nor-3-epi-4,5-~~dehydro~~ anhydro-6dEB (70a,b)
KOS016-152G	6	eryKR5 → rapDH/KR4 + eryAT6 → rapAT2 + eryKR6 → ΔrdX	2-nor-3-oxo-4,5-~~dehydro~~ anhydro-6dEB (71a,b) hemiketal (81a,b)
KOS016-152K	6	eryKR5 → ΔrdX + eryAT6 → rapAT2	2-nor-5-oxo-6dEB (44a,b)
KOS016-152I	6	eryKR5 → ΔrdX + eryAT6 → rapAT2 + eryKR6 → rapKR2	2-nor-3-epi-5-oxo-6dEB (72a,b)
KOS015-34	6	eryAT3 → rapAT2 + eryAT6 → rapAT2	2,8-bisnor-6dEB (47a,b)
KOS015-162	6	eryKR~~3~~ 2 → rapDH/ER/KR1 + eryKR5 → ΔrdX + eryAT6 → rapAT2	2-nor-5-oxo-11-deoxy-6dEB (73a,b)
***In Vivo* Ligation**			
KOS005-4	3	KR2 → ΔRdx	3-keto-TKL (2a,b)
KOS011-62	6	AT2 → rapAT2	10-nor-6dEB (17a,b)
KOS011-66	6	KR2 → rapDH/ER/KR1	11-deoxy-6dEB (21a,b)
KOS011-64	6	KR2 → rapDH/KR4	10,11-~~dehydro~~ anhydro-6dEB (22a,b)
KOS011-19	6	DH/ER/KR4 → rapDH/ER/KR1	6-epi-6dEB (23a,b)

Table 2			
Plasmid	Modules	Domain Substitution	Products (see Figure 6)
KOS011-21	6	DH/ER/KR4 → rapDH/KR4	6,7-~~dehydro~~ anhydro-6dEB (24a,b)
KOS011-22	6	DH/ER/KR4 → ΔRdx	7-oxo-6dEB (25a,b)
KOS011-74	6	KR6 → rapKR2	3-epi-6dEB (29a,b)
KOS011-25	6	KR6 → rapDH/KR4	2,3-~~dehydro~~ anhydro-6dEB (74a,b)
KOS011-13	6	KR6 → ΔRdx	3-oxo-6dEB (30a,b) 2-nor-3-oxo-6dEB(31a,b) spiroketal (77a,b) 2-nor-spiroketal (78a,b)

Please replace the paragraph beginning on page 41, line 16 with the following rewritten paragraph:

For the glycosylation reactions in the following examples, the title compound was used as a reagent. The conversions of paragraph (A) and (B) of this Example are shown in ~~Figure 9~~ Figure 10.

Please replace the paragraph beginning on page 42, line 14 with the following rewritten paragraph:

(A) A mixture of 6-deoxyerythronolide B (~~6-DEB~~ 6dEB) (15 mg, 39 umol) and 1-(2-mercaptopyrimidinyl)-2-O-methoxycarbonyl-(D)-desosamine (65 mg, 200 umol) was dried under vacuum, then placed under a nitrogen atmosphere. To this was added CH_2Cl_2 (1 mL), toluene (0.5 mL), and powdered 4A molecular sieves (50 mg), and the mixture was stirred for 10 minutes at ambient temperature. Silver trifluoromethanesulfonate (64 mg, 250 umol) was added and the reaction was stirred until LC/MS analysis indicated completion (18-20 hours). The mixture was filtered through anhydrous Na_2SO_4 and evaporated to yield crude product. The residue was dissolved in several drops of acetonitrile and loaded on a C-18 solid phase extraction cartridge (Whatman). Unreacted desosamine was removed by washing with 20% CH_3CN/H_2O and glycosylation products and the remaining macrolide aglycone were recovered by eluting with 100% CH_3CN. Final separation was carried out by HPLC using a semiprep C-18 column (10 mm X 150 mm) (CH_3CN/H_2O, 20% isocratic over 5 min, then 20% to 80% over 30 min). HPLC fractions were

checked by LC/MS and fractions containing the same product were combined. The solvent was removed under vacuum, yielding 8.4 mg of 5-O-[1-β-(2-O-methoxycarbonyl-(D)-desosaminyl)]-6-deoxy-erythronolide B (compound 86 in Figure 8) (36% yield). APCI-MS gives [M+H]+ = 602.

Please replace the paragraph beginning on page 43, line 1 with the following rewritten paragraph:

(B) 5-O-[1-(2-O-methoxycarbonyl-(D)-desosaminyl)]-6-deoxyerythronolide B (1-6 mg) from paragraph (A) was dissolved in 1 mL methanol, 0.2 mL H2O, and 0.2 mL triethylamine and kept at 70°C for 3 hours. Removal of the solvent under vacuum gave crude product. This was dissolved in a few drops of ~~CH3CN~~ CH_3CN and applied to a Whatman C18 solid phase extraction cartridge. The column was washed with 25 mL of 20% ~~CH3CN~~ CH_3CN in water, then the product was eluted with 100% ~~CH3CN~~ CH_3CN. Evaporation of the solvent gave 5-O-(1-β-(D)-desosaminyl)-6-deoxyerythronolide B (compound 87 in Figure 8) in quantitative yield. APCI-MS gives [M+H]+ = 544.

Please replace the paragraph beginning on page 43, line 25 with the following rewritten paragraph:

Preparation of 5-O-[1-β-(2-methoxycarbonyl-(D)-desosaminyl)]-3,6-dideoxy-3-oxoerythronolide B (~~Compound 83~~ Compound 90 in Figure 8) and 5,11-bis-(O-[1-β-(2-methoxycarbonyl-(D)-desosaminyl)])-3,6-dideoxy-3-oxoerythronolide B (Compound 92 in Figure 8).

Please replace the paragraph beginning on page 43, line 29 with the following rewritten paragraph:

Treatment of 3,6-dideoxy-3-oxoerythronolide B (6 mg) as described in Example 9(A) gave 5-O-[1-β-(2-O-methoxycarbonyl-(D)-desosaminyl)]-3,6-dideoxy-3-oxoerythronolide ~~B (compound 83~~ B (compound 90 in Figure 8) in 44% yield. APCI-MS gives [M+H]+ = 600. A second product, 5,11-bis-(O-[1-β-(2-O-methoxycarbonyl-(D)-desosaminyl)])-3,6-dideoxy-3-oxoerythronolide B (compound 92 in Figure 8), was also isolated from this mixture in 26% yield; APCI-MS gives [M+H]+ = 815.

Please replace the paragraph beginning on page 44, line 8 with the following rewritten paragraph:

Treatment of 5-O-[1-β-(2-methoxycarbonyl-(D)-desosaminyl)]-3,6-dideoxy-3-oxoerythronolide B of Example 11 as described in Example 9(B) gave 5-O-(1-β-(D)-desosaminyl)-3,6-dideoxy-3-oxoerythronolide B ~~of Example 11~~ (compound 91 in Figure 8) in quantitative yield. APCI-MS gives [M+H]+ = 542.

Please replace the paragraph beginning on page 44, line 22 with the following rewritten paragraph:

Treatment of (8R,9S)-10-deoxy-8,9-dihydro-9-hydroxy-8-methylmethymycin (12 mg) according to the procedure of Example 9(A) yielded 2'-O-methoxycarbonyl-(8R,9S)-10-deoxy-8,9-dihydro-9-hydroxy-8-methylmethymycin (~~compound 83~~ compound 82 in Figure 8) (34%); APCI-MS gave [M+H]+ = 544. A second product, 3,9-bis-(O-[1-β-(2-O-methoxycarbonyl-(D)-desosaminyl)])-(8R,9S)-10-deoxy-8,9-dihydro-9-hydroxy-8-methylmethonolide (compound 84 in Figure 8), was also isolated from this mixture (33%); APCI-MS gave [M+H]+ = 759.

Please replace the paragraph beginning on page 45, line 18 with the following rewritten paragraph:

A sample of 14,15-dehydro-6-deoxyerythronolide B (0.75 mg) from Example 7 was dissolved in 0.6 mL of ethanol and diluted to 3 mL with sterile water. This solution was used to overlay a 3 day old culture of *Saccharopolyspora erythraea* WHM34 (eryA) grown on a 100 mm R2YE agar plate at 30°C. After drying, the plate was incubated at 30°C for 4 days. The agar was chopped and extracted 3 times with 100 mL portions of 1% triethylamine in ethyl acetate. The extracts were combined and evaporated. The crude product was purified by preparative HPLC (C18 reversed phase, water-acetonitrile gradient containing 1% acetic acid). Fractions were analyzed by mass spectrometry, and those containing pure 14,15-dehydroerythromycin A (compound 95 in ~~Figure 8~~ Figure 9) were pooled, neutralized with triethylamine, and evaporated to a syrup. This was dissolved in water and extracted 3 times with equal volumes of ethyl acetate. The organic extracts

were combined, washed once with saturated aqueous ~~NaHCO3~~ $NaHCO_3$, dried over ~~Na2SO4~~ Na_2SO_4, filtered, and evaporated to yield 0.15 mg of product. APCI-MS gives [M+H]+ = 733.

Please replace the paragraph beginning on page 46, line 2 with the following rewritten paragraph:

Preparation of 14-oxo-8,8a-deoxyoleandolide (Compound 108) and 8,8a-deoxyoleandolide-14-carboxylic acid (~~compound~~ Compound 109) and Derivatives Thereof

Please replace the paragraph beginning on page 46, line 4 with the following rewritten paragraph:

These compounds can be prepared through ozonolysis of 14,15-dehydro-6-deoxyerythonolide B (compound 94 of ~~figure~~ Figure 9).

Please replace the paragraph beginning on page 46, line 25 with the following rewritten paragraph:

All diketide thioesters were synthesized by a common procedure. Illustrated here is the synthesis of (2S,3R)-3-hydroxy-2-methyl-4-pentenoic acid N-acetylcysteamine thioester. Enantioselective syn-aldol condensations were performed according to the procedure of D.A. Evans ~~et al~~ *et al.*, *J Am Chem Soc* (1992) 114:9434-9453. Subsequent manipulations followed the general procedures of D.E. Cane ~~et al~~ *et al.*, *J Antibiotics* (1995) 48: 647-651.

Please replace the paragraph beginning on page 46, line 31 with the following rewritten paragraph:

The synthesis of [4S,3(2S,3R)]-4-benzyl-3-(3-hydroxy-2-methyl-4-pentenoyl)-2-oxazolidinone by aldol condensation between (4S)-N-propionyl-4-benzyl-2-oxazolidinone (1.17 g, 5.0 mmol) and acrolein (0.4 mL, 11 mmol) was performed as described by D.A. Evans ~~et al~~ *et al.*, *J Am Chem Soc* (1992) 114:9434-9453, yielding 0.72 g of the adduct (50% yield) after chromatography on SiO2(2:1 hexane/ethyl acetate).